suggests that this region acts to increase the sensitivity of the C2A domain to its environment, and as such may tune the responsiveness of the domain.

2677-Pos Board B369
Infrared Radiation Activates Mitochondrial Calcium Cycling in Neurons
Vicente Lumbreras, **Suhrud Rajguru**.
Biomedical Engineering, University of Miami, Coral Gables, FL, USA.
Pulsed infrared radiation (IR) elicits controllable intracellular calcium and electrical responses in excitable tissue. Application of focused optical stimuli to target single cell activation and even subcellular mechanisms may lead to a broad range of basic science and prosthetic applications. It is therefore important to characterize the interactions between ion channels, microdomains and organelles in the intracellular Ca^{2+} cycling and related events. Towards this goal, we analyzed whether pulsed IR modulates mitochondrial membrane potential, Ψm, activating intracellular Ca^{2+} cycling. Experiments were performed on cultured spiral and vestibular ganglion neurons isolated from p2-p3 rat pups. The neurons were loaded and incubated for 30 minutes with Δψm sensors JC-1 (1.5 μM) or rhodamine 123 (10 μM). Oligomycin A and FCCP were used as positive controls. Pulsed IR was delivered to the neurons using a 400 μm optical fiber connected to a Capella laser (λ = 1863 nm). Image sequences of the neurons under IR stimulation were collected using a confocal microscope. Image processing was done using ImageJ and Matlab to study fluorescence intensity changes proportional to a stimulus. We measured the fluorescence intensity of the probes at the baseline level and after the application of IR stimulus. The neurons responded with a pulse-by-pulse response in rhodamine 123 fluorescence synchronized with the low frequency IR pulses applied. JC-1 capable of entering selectively into mitochondria reversibly changed its fluorescence emission after IR stimulation. The change in fluorescence intensity with pulsed IR reflects the change in relative levels of Ψm. The changes were reversible and matched the depolarization of mitochondria produced by positive controls. Results suggest that pulsed IR stimulus can potentially be utilized to study spatial Ca^{2+} buffering and the mitochondrial regulation of spatio-temporal properties of intracellular Ca^{2+} waves. Funded by NIH NIDCD R01DC011481.

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Cellular Mechanisms of Oxygen Sensing in Astrocytes
Plamena R. Angelova, Andrey Y. Abramov, Alexander V. Gourine.
University College London, London, United Kingdom.
In this study we used fluorescence imaging techniques to characterize the effects of an acute short-term hypoxia on intracellular [Ca^{2+}] signalling in primary cultures of astrocytes from the rat brain stem, midbrain, hippocampus and cortex. It was found that astrocytes respond with strong elevation in intracellular [Ca^{2+}] when PO2 in the incubation media decreases below 15 mmHg. Removal of external Ca^{2+} had no effect on hypoxia-evoked [Ca^{2+}]i signalling in astrocytes, while application of SERCA inhibitor thapsigargin completely blocked the responses. Cellular mechanism of PO2 sensing involves activation of the PLC pathway as demonstrated in the experiments showing blockade of the hypoxia-induced responses by various inhibitors of PLC downstream processes (U73122, 2-APB or Xestospongine C). Hypoxia-induced [Ca^{2+}]i responses were found to be completely blocked by depolarization of astroglial mitochondria using uncoupler FCCP and, therefore, appear to be dependent on the mitochondrial membrane potential. Ca^{2+} responses induced in astrocytes by decreases in PO2 lead to fusion of ATP and polyphosphate containing vesicular compartments. These data indicate that astrocytes are sensitive to physiological changes in PO2 and this sensitivity may have a functional significance in the control of local blood flow and the neuronal activity.

2679-Pos Board B371
Alpha-Synuclein Modulates [Ca^{2+}]c of Neurons and Astrocytes that Trigger Cell Death
Plamena R. Stroh[1], Marthe H.R. Ludtmann[1], Nicholas W. Wood[1], David Klenerman[2], Sonia Gandhi[1], Andrey Y. Abramov[1].
[1]UCL Institute of Neurology, London, United Kingdom, [2]Department of Chemistry, Cambridge University, Cambridge, United Kingdom.
Alpha-synuclein is a protein expressed abundantly in the brain that has been proposed to have multiple physiological functions as signal transduction, vesicular trafficking, synaptic behaviour, regulation of oxidative stress and mitochondrial function. However, when misfolded and aggregated, it forms intracellular inclusions (Lewy bodies) that are the hallmark of Parkinson's disease.
We used digital imaging technique to explore effects of alpha-synuclein on cytosolic calcium levels with Fura-2 indicator. We found out that the monomeric, as well as the aggregated form of alpha-synuclein (oligomers) evoked a [Ca^{2+}]c of primary neurons and astrocytes after delay ~5 min.
The [Ca^{2+}]c response to monomers and oligomers was entirely dependent on external calcium, and neither U73122, an inhibitor of PLC, nor the SERCA inhibitor Thapsigargin had any effect on the [Ca^{2+}]c signals. The response was not affected by inhibitors of either ionotropic or metabotropic glutamate receptors, including CNQX, MK-801 or (S) MCPG.
Further, we investigated using confocal live imaging whether this calcium signal contributed to alpha-synuclein induced neurotoxicity. We assessed the number of cells undergoing apoptosis within 30 min upon activation of caspase-3 (NucView 488) with oligomeric alpha-synuclein but not with monomers. Omission of the calcium from the extracellular recording medium (HBSS) prevented apoptotic cell death.
Additionally, pre-incubation of astrocytes and neurons from primary co-culture with oligomeric alpha-synuclein induced necrotic cell death within 12 h. as assessed with Hoechst/Propidium Iodide staining. However, inhibition of the alpha-synuclein-induced calcium signal in astrocytes and neurons by Ca^{2+}-free medium significantly reduced necrotic cell death by oligomers. Thus, monomeric and oligomeric alpha-synuclein evokes a [Ca^{2+}]c signal in neurons and astrocytes which could be a trigger for neurotoxicity.
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2680-Pos Board B372
Parvalbumin Tunes Spike-Timing and Efferent Short-Term Plasticity in Striatal Fast Spiking Interneurons
David Orduz[1], Patrick Bischop[1], Beat Schwaller[2], Serge N. Schiffmann[1], **David Gall**[1].
[1]Université Libre de Bruxelles, Bruxelles, Belgium, [2]University of Fribourg, Fribourg, Switzerland.
Striatal fast spiking interneurons (FSI) modulate the output of the striatum by synchronizing medium-sized spiny neurons (MSN). FSI are the only striatal neurons to express the calcium-binding protein parvalbumin (PV). This selective expression of PV raises questions about the functional role of this calcium buffer in controlling FSI calcium dynamics, and, consequently, the FSI spiking mode and neurotransmission. To study the functional involvement of FSI in striatal microcircuit activity and the role of PV in FSI function, we performed perforated patch recordings on EGFP-expressing FSI in brain slices from control and PV-/- mice. Our results revealed that PV-/- FSI fired more regularly and were more excitable than control FSI by a mechanism in which calcium buffering is linked to spiking activity as the result of the activation of small conductance (SK) calcium-dependent potassium channels. A modeling approach of striatal FSI supports our experimental results. Furthermore, PV deletion modified frequency-specific short-term plasticity at inhibitory FSI to MSN synapses. Our results therefore support the hypothesis that in FSI, PV is crucial for the fine-tuning of the temporal responses of the FSI network and for the orchestration of MSN populations. This, in turn, may play a direct role in the generation and pathology-related worsening of motor rhythms.

2681-Pos Board B373
Fractional Binding: A Molecular Analog-To-Digital Converter in Ca^{++} Regulated Vesicle Differentiation
Dimitrios Stamou, Kadla Rosholm, Ian Allen, Sune Christensen, Nicky Ehrlich, Jannik Larsen, Vadym Tkatch.
Bio-Nanotech Lab, Univ Copenhagen, Copenhagen, Denmark.
A critical feature of neurotransmission is that only a subset of vesicles react at a given Ca^{++} pulse. To achieve such a fractional response the synapse utilises a number of complex spatio-temporal and biochemical mechanisms that are still under debate but collectively appear to create distinct vesicle pools. We currently ignore whether differentiation of seemingly identical vesicles exists also for membrane trafficking vesicles.
We recently established that vesicles within an ensemble display large compositional inhomogeneities(1). Annexins, as well as other Ca^{2+}-dependent membrane binding proteins, are known to bind specific phospholipid components in cellular membranes(2). Here we investigated how the unique capability of Ca^{2+}-regulated membrane-binding proteins to bind certain lipids enables them to discriminate between single vesicles based on their lipid compositional heterogeneity. We employed human Annexin A5 (AnxA5), which is widely used as a model system for the membrane binding properties of the Annexin superfamily(2), and our previously described single vesicle assay(3, 4). We demonstrate that heterogeneities in the lipid composition of individual vesicles within a population, in conjunction with Ca^{2+} concentration, regulate the fraction of vesicles recruiting Annexins with digital precision. This is a recurrent phenomenon observed in multiple vesicle samples and for different Ca^{2+} sensing proteins. Thus, we anticipate that the cell utilize this feature to translate analog Ca^{2+} influxes into a selective digital targeting of vesicle sub-populations within the ensemble for specific functions.